

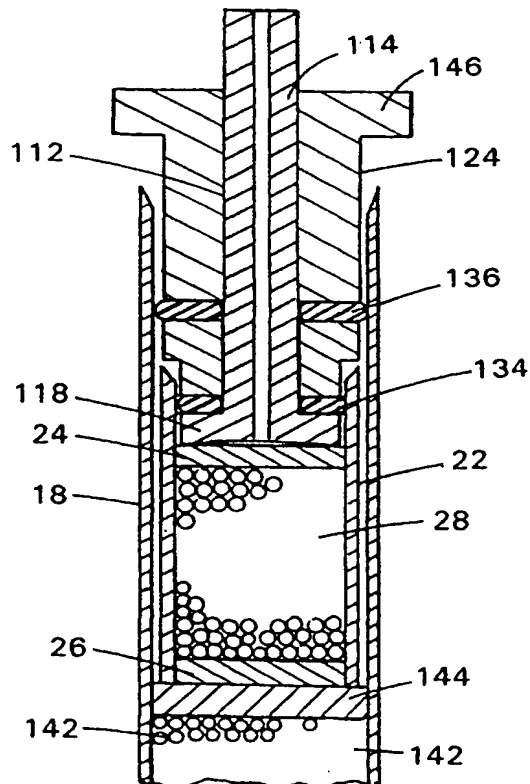
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|---|--|--|
| (51) International Patent Classification ⁶ : B01D 15/08 | | (11) International Publication Number: WO 00/10676 |
| A1 | | (43) International Publication Date: 2 March 2000 (02.03.00) |
| (21) International Application Number: PCT/US99/16010 (22) International Filing Date: 14 July 1999 (14.07.99) (30) Priority Data: 09/137,278 20 August 1998 (20.08.98) US (71) Applicant: DYAX CORPORATION [US/US]; One Kendall Square, Building 600, 5th floor, Cambridge, MA 02139 (US). (72) Inventors: HARGRO, Ivan; 2653 Hydraulic Road, Charlottesville, VA 22901 (US). HORSMAN, Jeffrey, A.; 1033 Wintergreen Lane, Charlottesville, VA 22903 (US). RAHN, Peter, C.; 2 Sand Trap Terrace, Palmyra, VA 22963 (US). VANDAVELAAR, Peter, C.; 1861 Wicker Woods Drive, Maidens, VA 23102 (US). (74) Agent: BOOTH, William, E.; Fish & Richardson, P.C., 225 Franklin Street, Boston, MA 02110-2804 (US). | | (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> |

(54) Title: MODULE AND METHOD FOR INTRODUCING A SAMPLE INTO A CHROMATOGRAPHY COLUMN

(57) Abstract

A chromatography sample module (16) including a flow-through member having an inlet and an outlet, chromatography media (28) within the flow-through member, and a sample carried on the media (28). The module (16) can fit within a chromatography column (18), and a plurality of modules (16) can be arranged in an array in a rack to facilitate processing of multiple samples.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | | | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mauritania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Malawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Congo | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | NZ | New Zealand | | |
| CM | Cameroon | | Republic of Korea | PL | Poland | | |
| CN | China | KR | Republic of Korea | PT | Portugal | | |
| CU | Cuba | KZ | Kazakstan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |

- 1 -

MODULE AND METHOD FOR INTRODUCING A SAMPLE
INTO A CHROMATOGRAPHY COLUMN

5 Background of the Invention

The invention relates to introducing a sample into a chromatography column.

Liquid chromatography is a technique for separating the individual compounds that exist in a subject sample. In employing the technique, the subject sample is carried in a liquid, called a mobile phase. The mobile phase carrying the subject sample is caused to migrate through a media, called a stationary phase. Different compounds will have differing rates of migration through the media, which effects the separation of the components in the subject sample. Liquid chromatography is commonly performed with reusable columns or with disposable cartridges, both of which are usually cylindrical, in which the media bed is bounded axially by porous plates, or plates containing defined flow paths, through which the mobile phase will flow. (See U.S. Pat. No. 4,250,035 to McDonald et al. and U.S. Pat. No. 5,601,708 to Leavesley)

When chemists optimize liquid chromatographic separations conditions, they may need to dissolve the sample mixture in a dissolution solvent which may be nonideal for elution. This can result in poor separation and poor recovery of desired components.

One solution to this problem is to pre-absorb the sample onto a media prior to chromatography. This involves dissolving the sample mixture in a suitable solvent and adding an amount of a dry media (usually similar to the media being used for the separation) to this solution. The dissolution solvent is then evaporated off, usually using a rotary evaporator, leaving the sample mixture dry, and absorbed to the

media. The pre-absorbed media is then placed at the head of a pre-packed glass, metal or plastic chromatography column, and the optimized chromatographic solvent would flow through the pre-absorbed media and then through the column of separation media. This method has the potential hazard of the operator coming into contact with the dry powdery media both before and after the addition of the sample. This method also can lead to poor separations and recovery.

Summary of the Invention

In one aspect, the invention features, in general, a chromatography sample module including a flow-through member having an inlet and an outlet and chromatography media within the flow-through member. A sample is added to the media, and the module, with the sample carried therein, can then be connected to a separation column.

Preferably the chromatography sample module is a tubular member that is sized to fit within the end of a chromatography column that is used for separation of the sample contained on the media in the module. Alternatively, the module can be connected to the chromatography separation column by a flow line. The sample in the dissolution solvent can be added to the sample module, and then the dissolution solvent can be evaporated. Alternatively, the sample in the dissolution solvent can be added to the sample module as a liquid without evaporation.

In another aspect the invention features a rack of sample modules arranged in an array.

Embodiments of the invention may include one or more of the following advantages. The samples can be easily introduced into separation columns. Various solvents can be used for separation and dissolution of the sample, permitting optimization of the separation procedure. Samples are easily preprocessed, and the

- 3 -

operator is not exposed to the media before or after adding the sample. A large number of samples can be prepared for processing at one time, facilitating the carrying out of multiple separations at one time.

5 Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

Brief Description of the Drawings

10 Fig. 1 is a schematic diagram of a chromatography system according to the invention.

Fig. 2 is a vertical sectional view of a chromatography sample module used in the Fig. 1 system.

Fig. 3 is a plan view of a rack containing a plurality of the Fig. 2 sample modules in an array.

15 Fig. 4 is an elevation of the Fig. 3 rack and modules.

Fig. 5 is a vertical sectional view showing the Fig. 2 sample module in position between a sealing head and a chromatography column used in the Fig. 1 system prior to assembly.

Fig. 6 is a vertical sectional view showing the Fig. 5 components in an assembled and sealed state.

Description of the Preferred Embodiments

Referring to Fig. 1, there is shown chromatography system 10 which includes a source of solvent 12, pump 14, 25 sample module 16, liquid chromatography column 18, and sample collection vessel 20. In this system, the sample to be analyzed is preabsorbed onto media in sample module 16 prior to pumping solvent into module 16 and into 30 chromatography column 18 to perform the separation procedure.

Referring to Fig. 2, it is seen that sample module 16 includes cylindrical plastic tube 22, porous plates 24, 26 (made of inert plastic porous frits), and

chromatography media 28 (only partially shown in the figures) between porous plates 24, 26.

As appears from Figs. 5 and 6, sample module 16 is designed to fit within chromatography column 18 at the entrance thereof and to be sealably connected to the sealing head. Tube 22 is designed to fit within column 18 with minimal space between the two; in particular, there is 0.000" to 0.010" of radial clearance.

Sample module 16 can be filled with media that is the same as or is different from the media of chromatography column 18. The sample is dissolved in the required solvent and added to the top of sample module 16, where it is drawn into the media by capillary action. This dissolution solvent is then removed by placing sample module 16 in a vacuum chamber. Heat may also be applied.

After sample module 16 has dried, it can be placed directly inside separation column 18 so that the lower porous plate 26 is in intimate contact with the surface of the separation media or with a porous plate within the separation column on top of the separation media.

Alternatively, sample module 16 can be placed in a remote tube connected by a solvent line. Alternatively, the sample can be dissolved in a separation solvent (or a weaker solvent), and added to module 16. The wet module can then be loaded into the column or into a remote tube.

Examples of the types of complex samples where this technique has particularly advantageous use include synthetic organic reaction mixtures and natural product extracts, (e.g., from fermentation broths or plants). These samples often need to be dissolved in a solvent not compatible with the optimized separation solvent. Solvents are organized according to their "solvent

- 5 -

strength," where hexanes have a value close to zero, and methanol has a value of 0.95. Optimized separation eluents often have a lower solvent strength; e.g., hexane:ethylacetate 1:1 has a solvent strength of 0.295.

5 If the sample needs to be dissolved in a strong solvent such as methanol, there will be a solvent strength difference of 0.655 seen initially after loading the sample onto the column, and this will impair the separation of the sample. If the sample dissolved in
10 methanol is instead preadsorbed to the media in the sample module and dried, the sample will not face this impairment during separation.

Referring to Figs. 3 and 4, sample modules 16 can be supplied in racks 32, and a whole rack of sample
15 modules 16 can be efficiently prepared at one time rather than one at a time.

Figs. 5 and 6 show the placement of a module 16 in a column 18 and the sealing of the module 16 and column 18 to a sealing head used to deliver solvent. Sealing
20 head 110 has first head piece 112, second head piece 124, intermediate head piece 128, and first and second annular elastomeric sealing members 134, 136.

First head piece 112 has body 114 with longitudinal axis 116. First head piece 112 has
25 outwardly extending shoulder 118, and contact face 120. Part of contact face 120 has a slightly conical shape or other concavity. First head piece 112 defines flow path 122 along axis 116.

Body 114 of first head piece 112 fits slidably
30 through central openings in second head piece 124, intermediate head piece 128, and first and second elastomeric sealing members 134, 136.

Second head piece 124 has outwardly extending compression member 146. Intermediate head piece 128 has
35 narrow portion 148 distal from second head piece 124.

First elastomeric sealing member 134 is adjacent to both shoulder 118 and narrow portion 148 of intermediate head piece 128. Second elastomeric sealing member 136 is adjacent to both intermediate head piece 128 and second head piece 124.

The outer diameter of tube 22 of sample module 16 is sized so that tube 22 fits into column 18. The inner diameter of tube 22 is sized so that it may slidably receive shoulder 118, first elastomeric sealing member 134, and narrow portion 148 of intermediate head piece 128.

Intermediate head piece 128, second elastomeric sealing member 136, and second head piece 124 are sized to fit slidably into column 18, having chamfered edges 140, filled with chromatography separation media 142, which is bounded axially by porous plates 144.

Referring to Fig. 6 seals are formed with the apparatus by inserting sample module 16 into column 18 so that second porous plate 26 abuts first porous plate 144. Referring to Fig. 5, sealing head 110 is then inserted into column 18 and tube 22 of sample module 16, so that shoulder 118, first elastomeric sealing member 134, and narrow portion 148 are within tube 22, and contact face 120 abuts porous plate 24. Sealing head 110 extends far enough into column 18 so that second elastomeric sealing member 136 opposes the inner surface of column 18.

Downward compressive force applied to outwardly extending compression member 146 causes second head piece 124 to slide relative to first head piece 112 and transmits compressive force to second elastomeric sealing member 136, intermediate head piece 128, first elastomeric sealing member 134, shoulder 118, porous plate 24, sample module media 28, porous plate 26, porous plate 144, and separation media bed 142. The compressive force causes first and second elastomeric sealing members

- 7 -

134, 136 to expand radially so that first elastomeric sealing member 134 forms a seal with tube 22, and second elastomeric sealing member 136 forms a seal with column 18.

5 The seals are released by relaxing or removing the downward force to second head piece 124, thereby reducing the compressive force on the components of sealing head 110 and reducing the radial expansion of elastomeric sealing members 134, 136.

10 Preferably, tube 22 and column 18 are made of high-density polyethylene. However, the columns may be constructed of other materials, including glass or stainless steel. Preferably, elastomeric sealing members are made of a fluorocarbon polymer, such as that sold
15 under the trade name CHEMRAZ.

Other embodiments of the invention are within the scope of the following claims.

What is claimed is:

1. A chromatography sample module comprising a flow-through member having an inlet and an outlet,
chromatography media within said flow-through
5 member, and
a sample carried on said media.
2. A chromatography sample module comprising a tubular member that is sized to fit within the end of a chromatography column, said module having an inlet and an
10 outlet, and chromatography media within said tubular member.
3. The module of claim 2 further comprising a sample carried on said media.
4. The combination comprising
15 a chromatography column having a module receiving region at an inlet end thereof, and
a chromatography sample module located within said module receiving region, said module including a flow-through member having an inlet and an outlet, and
20 chromatography media within said flow-through member.
5. The module of claim 4 further comprising a sample carried on said media.
6. The module of claim 1, 3 or 5 wherein said sample has been absorbed onto said media.
- 25 7. The module of claim 1, 3, or 5 wherein said sample is dissolved in a solvent that is held within said module on said media.

8. A chromatography method comprising
 providing a chromatography sample module including
 a flow-through member having an inlet, an outlet, and
 chromatography media within said flow-through member,
 5 dissolving a sample in a solvent resulting in a
 dissolved sample,
 adding said dissolved sample to said media, and
 flowing solvent into said inlet and directing the
 effluent from said outlet to a chromatography column.

9. The method of claim 8 further comprising
 evaporating said solvent from said module after said
 adding and prior to said flowing.

10. The method of claim 8 or 9 further comprising
 placing said module in said chromatography column prior
 15 to said flowing.

11. The method of claim 8 or 9 further comprising
 placing said module in said chromatography column prior
 to said flowing, and providing a seal between said module
 and said chromatography column prior to said flowing.

12. The method of claim 8 wherein said providing
 includes providing a plurality of sample modules in an
 array in a support structure,

each said module including a flow-through member
 having an inlet, an outlet, and chromatography media
 25 within said flow-through member, and

wherein said adding includes adding dissolved
 samples to said media in said plurality of sample modules

13. Chromatography sample preparation apparatus
 comprising

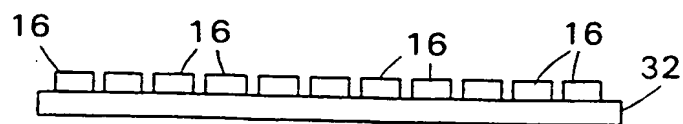
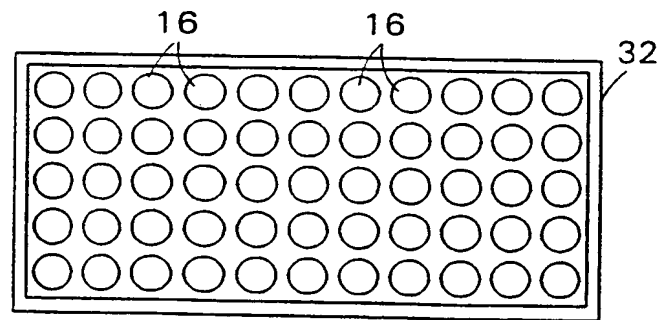
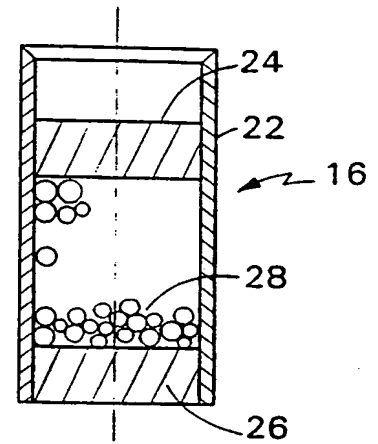
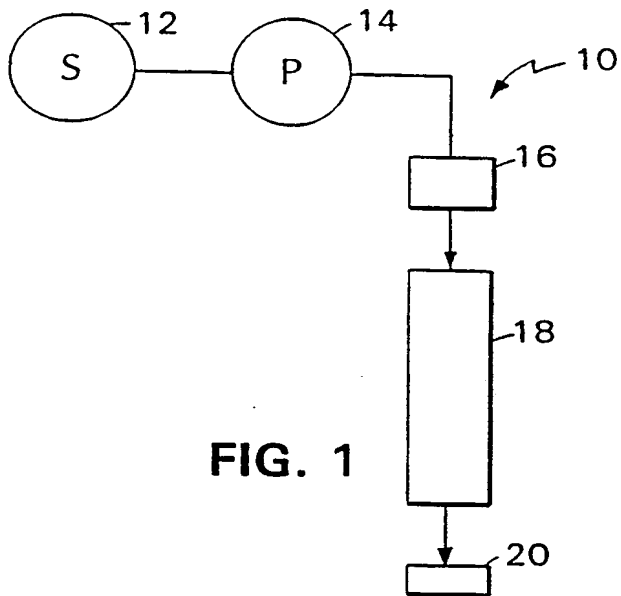
30 a plurality of chromatography sample modules, each
 said module including a flow-through member having an
 inlet, an outlet, and chromatography media within said
 flow-through member, and

a support structure supporting said plurality of
 35 modules.

14. The apparatus of claim 13 wherein said sample modules are adjacent to each other in said support structure.

15. The apparatus of claim 13, wherein said 5 samples are in an array in said support structure.

1/3



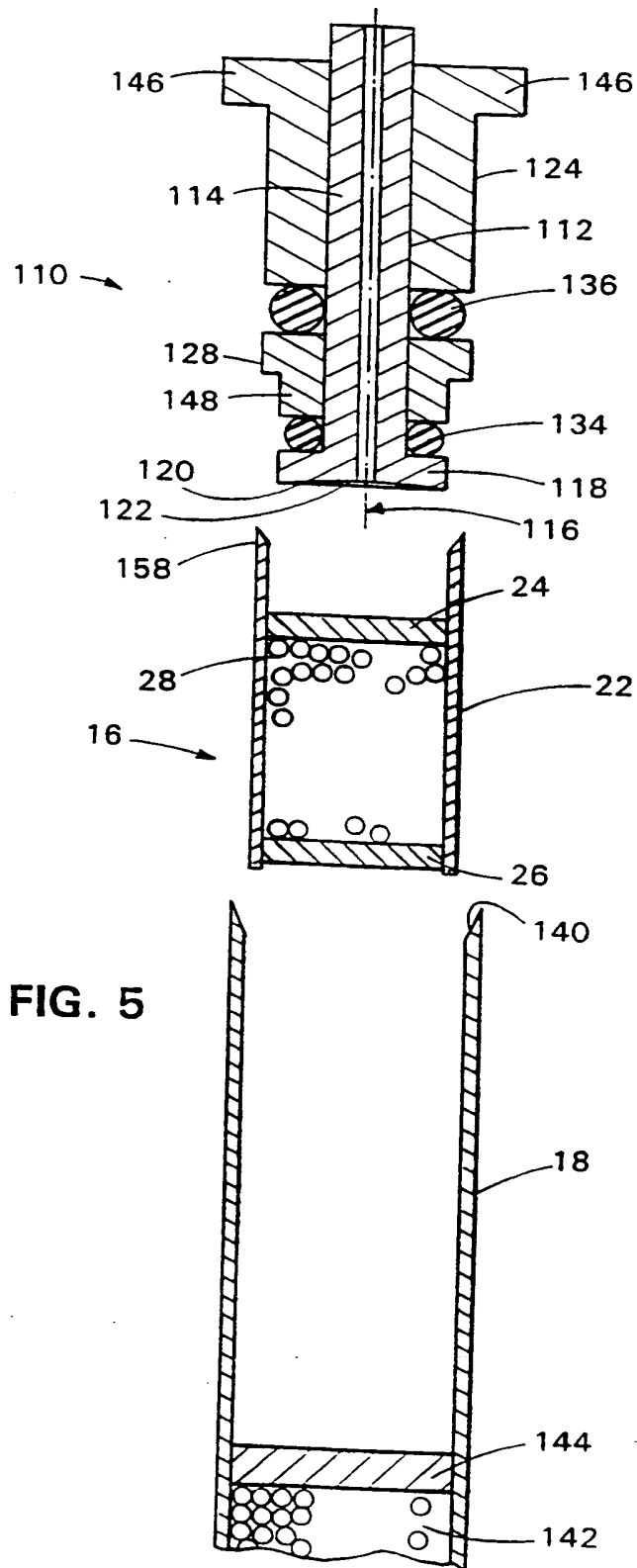


FIG. 5

3/3

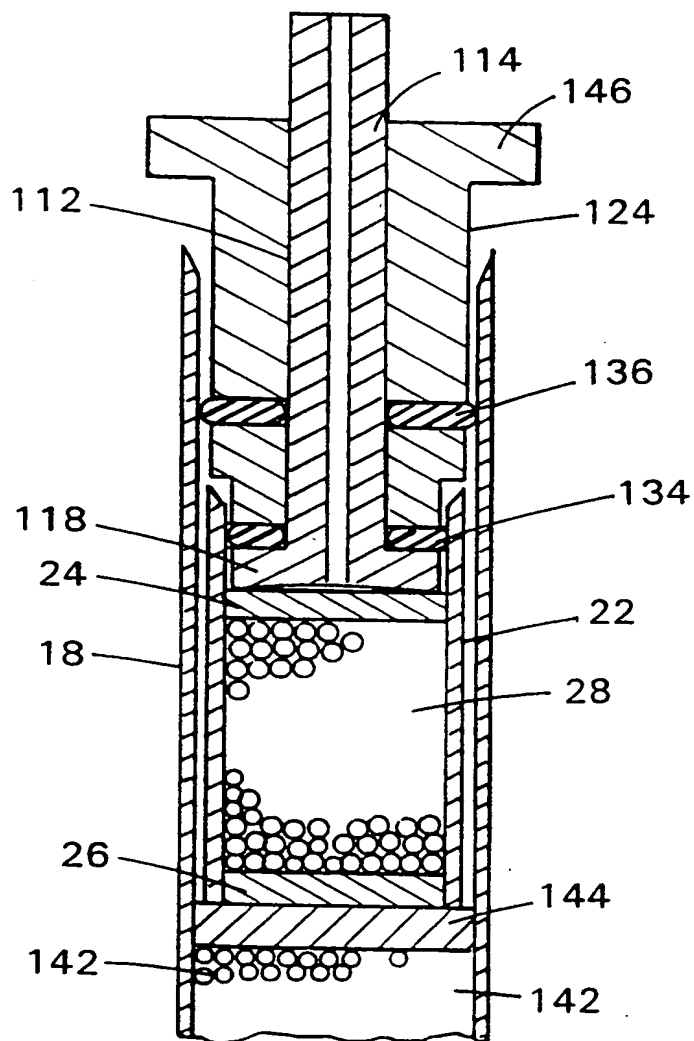


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16010

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : B01D 15/08

US CL : 210/656, 198.2, 541

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 210/635, 656, 659, 198.2, 541

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| Y | US 4,565,632 A (HATCH et al.) 21 January 1986, col. 3, lines 31-33. | 1-3 |
| Y | US 4,457,846 A (MONK) 03 July 1984, col.4, lines 62-65. | 1-15 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | |
|---|--|
| Special categories of cited documents: | |
| *A* document defining the general state of the art which is not considered to be of particular relevance | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| *E* earlier document published on or after the international filing date | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *O* document referring to an oral disclosure, use, exhibition or other means | *A* document member of the same patent family |
| *P* document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

24 AUGUST 1999

Date of mailing of the international search report

20 OCT 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ERNEST G. THERKORN

Telephone No. (703) 308-0362